

**Evaluation report of the bachelor thesis “Interaction of *Borrelia burgdorferi* spirochetes with the salivary glands of *Ixodes ricinus* and with tick cells in vitro observed by immunofluorescence microscopy” by Martin Strnad**

Martin Strnad submitted his bachelor thesis dealing with the role of outer surface proteins of *Borrelia* spirochetes, namely OspA and OspC, in interaction of the bacteria with tick cells. These questions are very actual and highly discussed in the scientific community.

The submitted work has 30 pages and contains all the essentials of bachelor thesis. Nine pages of introduction bring the reader into the topic and I appreciate the fact that Martin is not afraid to mention some of the controversial data published so far. Aims are defined simply and methodology is well described. Results are brought together in one table, followed by one-page discussion. Conclusion and list of abbreviations then go before the list of references containing 60 items. Pictures from the fluorescent microscope are aligned in the appendix.

As far as I am able to judge, the text is written in very good English and with the minimum of formal mistakes. Here just two comments: a) Please be more careful about the spacer next time; b) *Borrelia* should be written with capital B and in Italics.

I have following question and comments:

- 1) p.4, headline 1.1.: Are you sure that **Ixodida** are **genus**?
- 2) p.4: MYA (million years ago) is missing in the list of abbreviations.
- 3) p.7, chapter 1.4.: Are there any concrete and more recent proofs of the hypothesis that *B. burgdorferi* inhibits phagocytosis?
- 4) p.8, chapter 1.5.: In the last sentence you write that *Ixodes scapularis* is able to synthesize ligands which support the survival of *B. burgdorferi* within ticks. Which ligands do you mean?
- 5) Do you have any idea why ticks have both agonists and antagonists of histamine in their saliva?
- 6) Chapter 1.9. about the role of salivary glands seems to me too brief and superficial.
- 7) Table on the p. 14: What is the target for the primary antibody 4C12/C2 and what does anti-*Borrelia* medium mean?
- 8) p.15, chapter 3.2.2.: Why did you choose B31 (originated from North America) for the interactions with European tick cells?
- 9) p. 15 and 16: You have incubated the spirochetes with salivary glands at 34 °C and with tick cell line at 28 °C. Why?
- 10) By which mechanism(s) are spirochetes able to get inside the tick cell?
- 11) How would you explain the increase of *Borrelia*-positive cells (either inside of on the surface) after treatment with anti-OspA antibody?
- 12) As for the discussion, I think that it is too short even for the bachelors. I can feel some time press behind this part of the thesis. Unfortunately, the take-home message for me was that the anti-OspC antibody did not work. I would have appreciated a few words about why Martin did not try another antibody (a student-made anti-OspC monoclonal antibodies are available in the Prof. Kopecký freezer). It is also a pity that the IRE19 cells are not better defined.

Nevertheless, I believe that Martin has done the hard work, learnt a few of important laboratory procedures and he proved his bearing in the world literature. I recommend the thesis for the defense.

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